Egg Consumption is the Principal Risk Factor for Sporadic *Salmonella* Serotype Heidelberg Infections: A Case-Control Study in FoodNet Sites

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To determine risk factors for sporadic Salmonella serotype Heidelberg diarrheal disease, we conducted a population-based case-control study in 5 Foodborne Diseases Active Surveillance Network (FoodNet) surveillance areas in 1996–1997. Forty-four case patients and 83 control subjects matched by age and telephone exchange were asked about exposures during the 5-day period before onset of illness in the case patient. Risk factors for infection were evaluated using conditional logistic regression analysis. Eating eggs prepared outside the home remained the only significant risk factor for illness (matched odds ratio [MOR], 6.0; 95% confidence interval [CI], 1.2–29.6). The population-attributable fraction of S. Heidelberg infections associated with eating eggs prepared outside the home was 37%. Eliminating the risk associated with out-of-home egg consumption could substantially reduce the incidence of S. Heidelberg infections. Control measures to prevent S. Heidelberg infection should include advising consumers to avoid eating undercooked eggs and educating food handlers about proper egg handling and cooking.

Each year an estimated 1.4 million *Salmonella* infections and ~600 related deaths occur in the United States [1]. *Salmonella* serotype Heidelberg is the third most commonly reported infecting *Salmonella* serotype in the United States, behind serotypes Typhimurium and Enteritidis. From 1993 through 1997, an average of 2180 cases of *S.* Heidelberg infection were reported annually,

accounting for ~6% of all culture-confirmed *Salmonella* infections [2]. However, culture-confirmed illnesses represent only ~2.6% of all illnesses due to *Salmonella* infection, so the actual burden of illness from *S*. Heidelberg is estimated to be 84,000 cases of illness annually. Despite the frequency of these illnesses, relatively little is known about risk factors for *S*. Heidelberg infection or about potential methods of preventing illness.

Like other nontyphoidal salmonellae, *S.* Heidelberg appears to be largely a foodborne pathogen; there are only occasional reports of person-to-person [3, 4] or direct animal-to-person transmission [5]. Investigations of outbreaks of illness caused by *S.* Heidelberg have identified chicken [6, 7], pork (Centers for Disease Control and Prevention [CDC], unpublished data), eggs [8, 9], and cheddar cheese [10] as foods associated with illness. In addition, *S.* Heidelberg has been isolated from several foods, including chicken [11, 12] and pork [13, 14]; it has also been found on eggshells [15] and

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has been shown to grow in eggs [9]. Data from disease outbreaks and reports of organisms isolated from food and animals are helpful in determining potential sources for human illness, but it is difficult to extrapolate those data to account for risks to the entire population. Outbreaks of foodborne disease commonly involve food preparation mistakes in meals for large numbers of persons, such as those who are institutionalized or attend large group events. As with other foodborne infections, the number of Salmonella infections that are associated with outbreaks is small compared with the number of illnesses that are considered to be isolated or "sporadic." From 1988 through 1997, an annual average of 5379 cases of outbreak-associated Salmonella infection were reported to CDC, far fewer than the average annual number of 45,274 culture-confirmed cases of Salmonella infection reported to CDC during this time period [16, 17]. Therefore, evaluating the risk factors associated with sporadic illnesses is an important step toward a more complete understanding of what can be done to reduce the total burden of S. Heidelberg infection in the United States.

This is the first case-control study to determine risk factors for sporadic S. Heidelberg infections in the United States and the first to evaluate the proportion of illness attributable to these risk factors. This study was conducted by the Foodborne Diseases Active Surveillance Network (FoodNet), which is the principal foodborne disease component of the CDC's Emerging Infections Program [18]. FoodNet is a collaborative project among the CDC, the US Department of Agriculture, the US Food and Drug Administration, and the state health departments of the FoodNet surveillance areas (also known as "FoodNet sites": all of Minnesota and Oregon and selected counties in Connecticut, New York, Maryland, Georgia, Tennessee and California). These data will be of interest to persons concerned with the prevention and control of salmonellosis, and methods used in this study will be of interest to persons trying to understand and prevent illnesses that are predominantly sporadic but that also occur in episodic outbreaks.

METHODS

In 1996, the catchment areas of the FoodNet sites included Alameda and San Francisco Counties, California; Hartford and New Haven Counties, Connecticut; Clayton, Cobb, DeKalb, Douglas, Fulton, Gwinnett, Newton, and Rockdale Counties, Georgia; and the states of Minnesota and Oregon. The 1997 US Census Bureau population estimate for these areas was 14.3 million persons, or ~5% of the US population. Culture-confirmed cases of salmonellosis were ascertained by contacting, usually weekly, the clinical laboratories that processed stool samples in the catchment areas. Isolates were forwarded from the clinical laboratories to the state public health laboratory for serotyping. Personnel in each FoodNet site attempted to

contact and interview everyone with culture-confirmed illnesses due to *Salmonella* groups B or D within a 12-month period from May 1996 to August 1997. Everyone infected with *S.* Heidelberg was considered for the case-control study except in Minnesota, where every other case was selected. We obtained informed consent from participants and conducted the study in accordance with the guidelines for human research specified by the US Department of Health and Human Services.

Patients with culture-confirmed diarrhea due to infection with S. Heidelberg that was reported during this time period were eligible for inclusion in the study if they lived within the population catchment area and were English speakers. Because nontyphoidal salmonellosis in infants often occurs without symptoms of gastroenteritis, isolation of S. Heidelberg coincident with a compatible illness was sufficient to meet the inclusion criteria for infants. Patients were excluded from the study if the onset of illness was ≥10 days before the specimen that yielded S. Heidelberg was obtained from them, if they were part of a recognized illness outbreak for which the vehicle of transmission was identified, or if illness occurred ≤28 days after a culture-confirmed case of Salmonella infection in a household member. For each case, 2 healthy, age-matched controls were sought; age was matched according to the following age groups: <6 months, ≥ 6 and <12 months, ≥ 1 and <6 years, ≥6 and <12 years, ≥12 and <18 years, ≥18 and <40 years, ≥40 and <60 years, and ≥60 years. Controls were found by a progressive and sequential random-digit telephone number dialing method that was anchored on the phone number exchange of the case. To minimize potential bias, the 2 controls were recruited at different times of day. For example, if the first control was contacted during business hours, then the second control was matched from contact made during evening or weekend hours. Controls were eligible for inclusion if they resided in the population catchment area, were English speakers, and had neither diarrhea (defined as ≥3 loose stools in a 24-h period) nor a household member who had had a cultureconfirmed Salmonella illness in the 28 days before onset of illness in the matched case. All interviews were conducted by trained telephone interviewers using a standard questionnaire after obtaining informed consent; parents or guardians were interviewed for cases <12 years old.

Cases and matched controls were interviewed about potential exposures that occurred in the 5 days before the onset of diarrhea in the case, or before illness onset, if the case was an infant. Cases were contacted as soon as possible after culture confirmation and within 21 days after the date the specimen was obtained. All controls were interviewed within 7 days after the matched case was interviewed. For those persons with culture-confirmed *S.* Heidelberg infection who were not included in the case-control study, a descriptive case series was estab-

lished using data obtained through surveillance reports or the patient interview.

We gathered information on participants' demographic characteristics, clinical course of illness, medical history, and potential exposures. One hundred three questions about potential exposures and behaviors were used to gather detailed information regarding water consumption, animal exposure, meal preparation, site of meal consumption, food handling practices, and the preparation and consumption of specific produce, dairy, and animal products. For infants, questions were modified to assess age-appropriate foods and behaviors.

Data entry was conducted in each FoodNet site using Epi Info (CDC) and analysis was conducted using SAS software, version 6.12 (SAS Institute). Differences in proportions between enrolled and nonenrolled cases were evaluated using the χ^2 test. The difference in the median age of patients was examined using the Wilcoxon rank-sum test. Univariate and multivariable risk factor analyses were conducted using conditional logistic regression in the SAS software "Proc Phreg" procedure to assess food exposures of interest. Variables significantly associated with illness at a P value of <.1 in univariate analysis were included in a backward regression model that was examined for possible pairwise interaction at the significance level of .05 and then assessed for confounding. Population attributable fractions were calculated using matched odds ratios generated from conditional logistic regression [19]. These odds ratios are approximately equivalent to relative risk estimates. CIs were computed for model-adjusted exposure-specific attributable fractions using a jackknife procedure outlined by Kahn et al. [20].

RESULTS

Active surveillance. During the study period, 139 culture-confirmed cases of S. Heidelberg infection were ascertained. The annual incidence of culture-confirmed S. Heidelberg infection in the FoodNet catchment area was 0.96 cases/100,000 population (table 1); it varied by state and ranged from 0.74 in Connecticut to 1.13 in Minnesota, but the variation was not statistically significant (P = .18). Hospitalization was reported for 25 (21%) of the 122 patients for whom these data were available. There were no deaths reported among cases.

Case-control study. After omission of patients who were excluded by the selection process used in Minnesota, 112 persons were eligible for the case-control study; 83 (74%) were interviewed. The primary reasons for not being interviewed included being unreachable (67% of those not interviewed) and not having a home telephone (18%). Of the 83 patients interviewed, 51 (61%) met the inclusion criteria for the study. The primary reasons for exclusion from the study were being contacted >21 days after the culture sample was obtained (41%)

Table 1. Rate of isolation of *Salmonella* serotype Heidelberg by FoodNet site, 1996–1997.

Site	No. of cases of S. Heidelberg infection	1997 FoodNet population	Cases/100,000 population
California	21	2,103,374	1.00
Connecticut	12	1,617,341	0.74
Georgia	26	2,775,193	0.94
Minnesota	53	4,685,549	1.13
Oregon	27	3,243,487	0.83
All sites	139	14,424,944	0.96

and having no symptoms of diarrhea during the illness (24%). One or more controls were interviewed for 44 (86%) of the 51 interviewed cases that met the inclusion criteria: 44 patients and 83 controls were enrolled in the case-control study; 39 cases were matched to 2 controls, and 5 cases were matched to 1 control. The cases who were enrolled and the 39 patients infected with S. Heidelberg who were not enrolled were similar in all measured characteristics, except that patients not enrolled were less likely to report having had fever and were more likely to report being in the lowest income group (table 2). The median age for enrolled cases was 11.5 years (range, 2 months-57 years); 54% were female. The median age for controls was 18.5 years (range, 2 months-56 years); 55% were female. Cases and controls did not differ significantly in age (P = .59). No deaths were reported among the persons identified with S. Heidelberg infections.

In univariate analysis, risk factors significantly associated with S. Heidelberg infection included eating eggs cooked somewhere other than the infected person's home (matched odds ratio [MOR], 6.4; 95% CI, 2.1-19.4) (table 3). This included eggs that were cooked outside of the home in several ways: runny eggs (MOR, 12.2; 95% CI, 1.5-99.5), fried eggs (MOR, 9.1; 95% CI, 1.1-78.4), and scrambled eggs (MOR, 4.6; 95% CI, 1.4-14.4). Other risk factors included eating runny eggs cooked anywhere (MOR, 4.4; 95% CI, 1.2-16.4) or eating chicken cooked somewhere other than the person's home (MOR, 2.5; 95% CI, 1.0-5.9). Two risk factors identified in previously reported S. Heidelberg disease outbreaks, consumption of cheese and consumption of pork, were not associated with illness in this study. Neither were S. Heidelberg infections associated with self-reported food preparation habits such as hand washing or cutting board use, nor with exposure to live animals, water consumption, or demographic factors. Several foods were associated with an apparent lower risk of illness, including a variety of fruits and vegetables and foods of animal origin that were prepared at home. However, in multivariate analysis these factors were determined to be unrelated to disease status.

In multivariate analysis, we assessed which potential risk fac-

Table 2. Demographic and clinical characteristics of persons infected with *Salmonella* serotype Heidelberg according to their enrollment status in the FoodNet case-control study, 1996–1997.

Characteristic, by class	Enrolled patients (n = 44)	Patients not enrolled (n = 30)	Р	
Demographic				
Age in years, median (range)	11.5 (<1–57)	32 (<1–86)	.07	
Female sex	54	53	.95	
Race or ethnicity				
White	80	82	.77	
African American	16	11	.45	
Asian	5	5	.9	
Education				
Less than high school	9	15	.86	
High school graduate	65	51	.18	
College graduate	26	33	.41	
Residence				
Urban	52	53	.89	
Suburban	28	25	.87	
Rural or small town	21	25	.58	
Annual income				
<\$15,000	5	20	.03	
\$15,000-\$29,000	36	27	.43	
\$30,000-\$59,999	31	24	.37	
≥\$60,000	28	28	.92	
Clinical				
Fever	93	75	.04	
Vomiting	43	48	.62	
Cramps	85	87	.69	
Bloody stools	46	44	.87	
Hospitalized	21	26	.53	
Length of hospitalization, days, mean (range)	4 (2–10)	4 (1–14)		

NOTE. Data are no. (%) of patients, unless indicated otherwise.

tors were independently associated with illness. All risk factors identified as statistically significant in univariate analysis, as well as cheese and pork consumption, were included in an initial model. After evaluating for interaction and removing factors not associated with illness, we arrived at a final model that included 3 risk factors. This model included consumption of chicken prepared away from the home, because this has been previously found to be a risk factor in outbreaks of *S*. Heidelberg infection and in univariate analysis in this study. We also included consumption of runny eggs, because this is an identified risk factor for infection with *S*. Heidelberg and other *Salmonella* serotypes, such as *Salmonella* Enteritidis. In this analysis, we found that eating eggs prepared somewhere other than the person's home was the only risk factor associated with *S*. Heidelberg infection at the significance level of .05 (MOR,

6.0; 95% CI, 1.2–29.6). No statistical interactions were detected. Of all *S*. Heidelberg infections in this population, 39% (95% CI, 14%–64%) were attributable to eating eggs prepared outside the home. It is noteworthy that for the group of study participants as a whole, eggs prepared outside the home were more likely to be runny than were eggs prepared at home (8 ([5%] of 23 eggs cooked outside home compared with 8 [14%] of 56 eggs cooked at home; χ^2 test, 4.2; P = .04).

DISCUSSION

In this population-based case-control study we found that the principal risk factor for sporadic infection with *Salmonella* was consumption of eggs prepared somewhere other than the person's home. More than one-third of these eggs were reported to be runny, indicating that they were insufficiently cooked to kill contaminating organisms. Eggs are a known source of outbreaks of *S.* Heidelberg infection, but the contribution of eggs to sporadic illness has not previously been determined. These data indicate that control measures to decrease illness caused by eggs, especially those cooked outside the home, could substantially reduce the incidence of *S.* Heidelberg infection.

In the United States from 1987 to 1997, the annual number of reported cases of S. Heidelberg infection in humans has declined by 65% (from 6017 to 2104 cases) [21]. This may be due to a decline in poultry-associated infections following improvements in sanitation during poultry production and processing. The data presented here were collected after the decline in reports of S. Heidelberg infection had occurred and point strongly to eggs as the current leading risk factor. It is not known whether people acquire S. Heidelberg infections as a result of exposure to contaminated eggshells or by eating intact eggs with transovarian contamination (as can occur with S. Enteritidis). S. Heidelberg has been found in chicken manure, has been cultured from the shell surface of eggs, and has been shown to penetrate the shell, but it has also been found in the ovaries and peritoneum of egg-laying chickens [9, 12]. Egg consumption has also been found to be a risk factor for infection with other Salmonella serotypes, including Enteritidis and Typhimurium [22-24]. Consumption of eggs in the United States is common; average consumption was estimated to be 258 eggs per person per year in 2000 [25]. Consumption of undercooked eggs, such as runny eggs, is also relatively common. In a FoodNet study of food consumption practices, 18% of people reported eating runny eggs in the 5 days before the interview [26]; we found a similar proportion among controls in our study (13%). Runny eggs are not heated sufficiently to coagulate proteins in the yolk or white or to kill bacteria in the yolk [27].

Meals eaten out of the home have been an increasing part of the American diet since the 1970s [28, 29]. In restaurants

Table 3. Univariate risk factors for Salmonella serotype Heidelberg infection, FoodNet case-control study, 1996–1997.

	Proportion (%) of subjects ^a		
Food eaten, where prepared	Cases	Controls	MOR ^b (95% CI)
Runny egg, outside home	7/36 (19)	1/71 (1)	12.2 (1.5–99.5)
Fried eggs, outside home	5/38 (13)	1/72 (1)	9.1 (1.1–78.4)
Any eggs, outside home	15/37 (41)	7/72 (10)	6.4 (2.1–19.4)
Scrambled eggs, outside home	10/37 (27)	5/71 (7)	4.6 (1.4–14.7)
Runny eggs, anywhere	10/32 (31)	6/64 (9)	4.4 (1.2–16.4)
Chicken, outside home	19/33 (58)	22/67 (33)	2.5 (1.0-5.9)
Chicken, at home	15/36 (42)	54/70 (77)	0.13 (0.04–0.40)
Eggs, at home	14/36 (39)	33/67 (49)	0.6 (0.3–1.4)
Pork, anywhere	7/36 (19)	26/71 (37)	0.44 (0.2-1.4)
Shredded cheese, anywhere	9/39 (23)	16/68 (24)	1.1 (0.4–2.7)

^a No. of persons who ate the food/total number of respondents (%).

where eggs are served, a common practice is to mix, or "pool," large numbers of uncooked eggs and then portion them out when cooking individual meals such as omelets or scrambled eggs. This practice has the potential to spread the bacterial contents of a few contaminated eggs among many servings. If these pooled eggs are held unrefrigerated or are systematically undercooked, the number of persons exposed can be greatly increased. Although we did not specifically determine where consumption of eggs outside the home occurred, it is likely that a high proportion of the eggs eaten outside the home were eaten in commercial food establishments, particularly restaurants. Thus instituting safer food preparation practices in commercial kitchens could probably reduce much of the risk associated with runny egg consumption.

We believe that the participants in this case-control study are representative of persons in the FoodNet sites with known S. Heidelberg infections, because they were identified through well-established laboratory-based public health reporting mechanisms that have been shown to be highly sensitive for detecting culture-confirmed cases [30]. Thus, all known S. Heidelberg infections in the FoodNet population should have been identified. Although not all cases were enrolled in the study, enrollees and nonenrollees were drawn from the same population base, and the 2 groups were similar with respect to sex, age, and clinical characteristics. By enrolling cases and controls during the same time period, we are able to estimate the relative risk due to exposures of interest by use of MORs and to compute population-attributable fractions applicable to the >14 million persons in these FoodNet sites [19, 20, 31, 32]. In doing so we assumed that bias was absent in the selection of cases and controls, that the potential cases comprise a closed cohort, and that no additional risks would be introduced if the risks due to eating eggs prepared outside the home were eliminated.

This is, to our knowledge, the first epidemiologic study of sporadic S. Heidelberg infections. Such a study would have been difficult before the establishment of FoodNet, because potential study participants are widely dispersed. In 1998, <5% of culture-confirmed Salmonella infections were associated with recognized outbreaks [33]. Further, risk factors identified in outbreaks may be different from those responsible for sporadic illnesses. Because many foodborne infections are not associated with outbreaks, data from studies of sporadic illness are particularly useful in developing prevention or control programs. For example, although contaminated poultry is believed to be a common source of Campylobacter jejuni infection [34], reported outbreaks of C. jejuni infection due to consumption of these foods are relatively rare, compared with outbreaks due to consumption of contaminated water or raw milk or due to infection from household pets [35]. Efforts to reduce the number of C. jejuni infections that are based solely on risk factors identified from disease outbreaks might not account for the principal risk factor, contaminated poultry. In our investigation, we evaluated risk factors for S. Heidelberg infection that were previously reported through outbreak investigations (consumption of cheese, pork, or chicken) but did not find them to be associated with sporadic illness. However, our study may not have had adequate statistical power to detect weak associations with common food exposures, such as consumption of cheese, pork, or chicken. In addition, the epidemiology of S. Heidelberg infection appears to have changed, and data from prior outbreaks may not reflect current risk factors. Therefore, these data should not be interpreted to indicate that food items other than eggs do not contribute to sporadic illnesses due to S. Heidelberg, although they do indicate that the primary focus of efforts to prevent S. Heidelberg infection should be reduction of the risks associated with egg consumption.

^b Matched odds ratios (MOR) were obtained by conditional logistic regression.

Activities that could reduce illness associated with egg consumption include educating consumers and commercial food preparers about the potential for bacterial contamination of eggs. Consumers, especially children, the elderly, pregnant women, and immunocompromised persons, should avoid eating undercooked eggs. However, undercooked eggs are often included in popular recipes. In a recent evaluation of recipes that included eggs in 14 popular cookbooks, 10 books (71%) had recipes that included unheated eggs in the final product, but only 4 of these books cautioned the reader about the risks associated with this practice (CDC, unpublished data). For those recipes likely to contain undercooked eggs (e.g., Caesar salad, Hollandaise sauce, and homemade ice cream), pasteurized eggs can be substituted for fresh eggs. Consumers and commercial food preparers should be aware that cross-contamination from uncooked eggs to foods that are ready to eat can occur via inadequately washed hands, utensils, containers, and surfaces. The use of pooled eggs should be restricted to small batches that are used immediately after pooling. Commercial food establishments and regulators should take steps to ensure that eggs are cooked thoroughly and that consumers are made aware of the risks of illness, should they insist upon undercooked eggs. Another measure that could reduce the risk associated with egg consumption is ensuring the cold storage of eggs from farm to table to slow the growth of bacteria that may be present on or within the egg.

Success in preventing and controlling illnesses caused by eggassociated *Salmonella* serotypes in the United States will depend on a multifaceted approach involving consumers, health care providers, public health personnel, cookbook writers and editors, restauranteurs, grocers, egg producers and distributors, and agricultural regulators. Future research needs to include the determination of the potential for internal and external contamination of eggs with *S.* Heidelberg and of ways to minimize contamination of eggs on the farm, at retail, and in the kitchen.

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